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AVIAN TUBERCULOSIS.*†

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Avian tuberculosis has not attracted especial attention in this country until quite recently. It was reported by Pernot¹ in 1900, but previously a disease had been described to which the term "tuberculosis" had been applied, although, as far as we are aware, no demonstration of the bacilli in lesions was made before the work of Pernot. Within recent years it has attracted greater attention, and is coming to be recognized as an economic problem in the poultry industry. It seems probable that the disease has spread rapidly within the last few years, due to the great increase in the sale of birds for breeding purposes and in the sale of eggs for hatching, and that this increase in avian tuberculosis has been the active factor in bringing the disease to the attention of experiment station workers. Tubercle bacilli were not found in any avian tissue submitted to this station for examination prior to 1906. During the last three years tubercular tissues have been received from many

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† Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

¹ Bull. 64, Oregon Exp. Sta.

sections of the state. From the information obtained in this and other ways it is apparent that avian tuberculosis is widespread in Wisconsin. A number of other stations have issued publications in which data concerning the distribution of the disease is given.¹ The number of inquiries answered in the poultry journals and in the agricultural papers, that without doubt refer to avian tuberculosis, is very considerable. All these facts indicate the widespread distribution of the disease in this country.

In Europe it was recognized before the discovery of the tubercle bacillus by Koch, and has long been a factor of importance, not only among farm poultry, but in the aviaries of the great zoölogical gardens. Koch and Rabinowitsch² have made an extensive study of the disease, using as their source of material the 459 birds that died in the Berlin garden from December, 1903, to August, 1905. Twenty-seven per cent were found to be tubercular. Of the 500 birds from the London garden, examined by Shattock, Seligmann, Dudgeon and Panthon³ 30 per cent were tubercular. The British Royal Commission on Tuberculosis has also studied in detail the characteristics of 14 cultures.⁴

The great losses occasioned by the disease in a limited time have been emphasized by Ward,⁵ Marshall,⁶ and Edwards.⁷ In two of the flocks studied by us, losses ranging from 33 to 50 per cent during less than one year were encountered. It seems probable, however, that such losses are exceptional, and that in most diseased flocks the losses are not sufficiently large to attract attention.

Characteristics of avian tuberculosis.—The birds showing visible symptoms of the disease are usually over one year old, but feeding experiments have shown that young chickens are easily infected. It is probable that under natural conditions it requires a number of months for the disease to make such progress in the individual bird as to become apparent. A rooster purchased by a poultry man from the station flock, which is free from tuberculosis, was placed in a flock subsequently found to be diseased. One year

¹ *Bull.* 161, California Exp. Sta., 1904; *Bull.* 193, Ontario Agri. Coll.; *Circular* 12, Michigan Exp. Sta., 1911.

² *Virch. Arch.*, 1907, Beihefte, 190, p. 246.

⁵ *Bull.* 161, Cal. Exp. Sta., 1904.

³ *Lancet*, 1907, 2, p. 1443.

⁶ *Circular* 12, Mich. Exp. Sta., 1911.

⁴ *Final Report*, Part II, Appendix, 1911, 4, p. 167.

⁷ *Bull.* 193, Ontario Agri. Coll., 1911.

later a postmortem examination showed that the bird was in the last stages of tuberculosis.

The first apparent symptom of the disease is the marked loss of flesh. The weight of some of the birds examined by us has been less than one-third that of the normal bird. The extreme to which the emaciation may go before death ensues is shown in Fig. 1, in which is presented a cross-section of the breast of a tubercular bird as compared with that of a healthy bird. Lameness is often

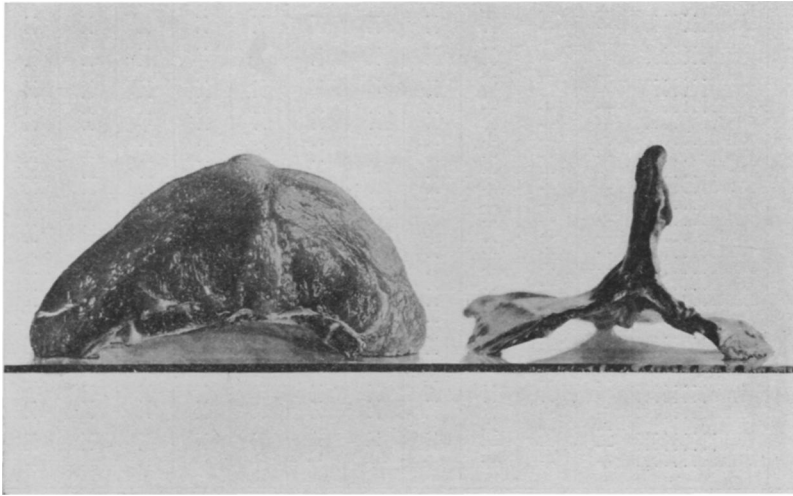


FIG. 1.—Extreme emaciation in avian tuberculosis. Cross sections of the breast of a healthy bird and a tubercular one.

noted. This condition may be due to diseased joints or to extensive changes in the viscera. The popular names, applied to the disease, such as “going light,” and “rheumatism” refer to these symptoms.

Avian tuberculosis is to be classed as a disease of the organs of the abdominal cavity rather than of the pleural cavity. This is shown in Table 1 in which is given the distribution of the lesions in the 29 fowls examined.

In Table 2, is presented a summary of the results obtained in our work as compared with those of Edwards,¹ Moore,² and Koch and Rabinowitsch.³

¹ *Bull.* 193, Ontario Agricultural College., 1911.

The Pathology of the Infectious Diseases of Animals, 1906, p. 189.

² *Op. cit.*

The figures with reference to lesions in the ovary are not accurate, except in our work and that of Edwards. No data was given by the other investigators as to the relative proportion of

TABLE 1.
DISTRIBUTION OF LESIONS IN VARIOUS ORGANS OF TUBERCULAR FOWLS.
The plus sign indicates the presence of lesions in a given organ.

Fowl	Died or killed	Liver	Spleen	Intes- tine	Kid- ney	Ovary	Mes- en- tery	Lungs	Pan- creas	Joints	Bones	Giz- zard
1	Killed.....	+	+	+
2	Killed.....	+	+
3	Killed.....	+	+
4	Killed.....	+	+	+
5	Killed.....	+	+	+
6	Killed.....	+	+	+
7	Died.....	+	+	+
8	Died.....	+	+	+
9	Killed.....	+	+	+
10	Died.....	+	+	+	..	+	+	..
11	Died.....	+	+	+
12	Killed.....	+	+	+
13	Killed.....	+	+	+
14	Killed.....	+	+	+
15	Died.....	+	+	..	+	+
16	Died.....	+	+	+	+
17	Died.....	+	+	+	+
18	Died.....	+	+
19	Died.....	+	+
20	Died.....	+	+	+
21	Died.....	+	+	+	+
22	Died.....	+	+	+	+
23	Killed.....	+	+	+	+	..
24	Killed.....	+	+	..	+	+
25	Killed.....	+	+
26	Killed.....	+	+
27	Died.....	+	+	+	+
28	Died.....	+	+	+	..	+	+
29	Killed.....	+	+	+	+	+	+	+	+

TABLE 2.
PERCENTAGE OF BIRDS SHOWING LESIONS IN VARIOUS ORGANS.

Organs Involved	Wisconsin	Edwards	Moore	Koch and Rabinowitsch
	Per cent	Per cent	Per cent	Per cent
Liver.....	97	99	76	85
Spleen.....	93	93	47	74
Intestine.....	60	61	35	41
Kidney.....	10	13	17	8
Ovary.....	14	13	6	4
Mesentery.....	17	21	23	..
Lungs.....	22	19	6	66
Bones.....	9	43	6	..

male and female birds examined. The work of Koch and Rabinowitsch was not confined to domestic fowls as in the case of the other investigators. The work of the German investigators has shown that probably all kinds of birds are susceptible to the disease.

The general appearance of the lesions has been described in many papers and texts. The tissue changes may be most extensive before death occurs. This was well shown in the case of Fowl 20, Table 1, in which the internal organs were badly diseased. The liver weighed 230 gms. and but little normal tissue remained in the spleen. The pronounced tissue changes, together with the enormous development of the bacilli in the lesions indicate the nontoxic nature of the disease.

The constant occurrence of tubercles on the walls of the intestine indicate that the organisms are eliminated in the feces and the

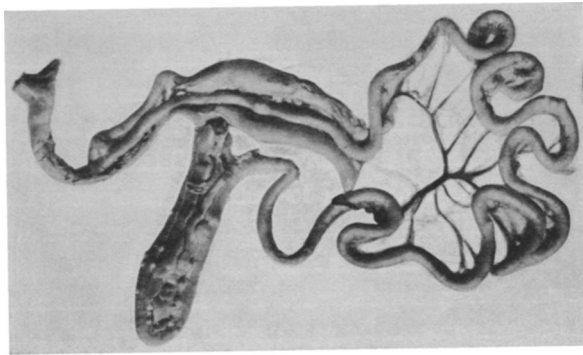


FIG. 2.—Tubercles on the intestines.

infection undoubtedly occurs by way of the alimentary tract. Weber and Bofinger¹ found it difficult to infect chickens by other methods than feeding. Intraperitoneal and intramuscular inoculation gave positive results only when large quantities of the culture were used. In our work intraperitoneal injections have always yielded positive results. A limited number of feeding experiments both with tubercular tissues and pure cultures have given positive results except in one case.

Rate of dissemination of avian tuberculosis.—An effort was made to obtain some evidence regarding the rate at which the disease may spread in a flock. For this purpose nine hens were obtained from a diseased flock. Four of the nine showed physical symptoms of tuberculosis. The above birds were placed with seven hens

¹ *Tuberculöse Arb. a.d. k. Gesundheitsamte*, 1904, 1, p. 83.

from a healthy flock. During the first month the birds were kept in a small room which was not cleaned during this period. One of the diseased birds died in 10 days. Postmortem examination showed tubercles on the intestines, and generalized tuberculosis of the abdominal cavity. Two others died in 26 and 46 days respectively. Both were extensively diseased and must have been eliminating tubercle bacilli. Of the remaining six hens from the tubercular flock which were killed one year later, five were found to be tubercular, but in none was the disease far advanced.

During the remainder of the year in which the healthy birds were associated with diseased ones, the flock was kept in a small colony house with a small yard. The house was not cleaned and the feed was scattered in the litter in order to make the exposure as extreme as possible. During the first two months of the experiment the exposure to infection was most complete, since the flock then contained birds in the last stages of tuberculosis. The first of the originally healthy hens was killed after eight months. The spleen and liver were tubercular and one nodule was found on one of the ceca. The remainder of the originally healthy hens were killed after having associated with the tubercular birds for about one year. Two of the six were tubercular, the others healthy. Thus three out of seven healthy hens acquired the disease. At the time they were killed the disease was not far advanced, and it is certain that the disease would not have progressed to a fatal termination in several months.

Transmission from flock to flock.—There would seem to be little doubt but that the diseased bird is the important agent in the spread of the disease from flock to flock. As shown in Table 1, the ovary is often involved. No examinations of eggs have been made by us. Mohler and Washburn examined a number of eggs laid by a tubercular hen and were able to demonstrate the presence of tubercle bacilli in one. More recently Higgins¹ reports on the bacilli in eggs from a diseased flock. Koch and Rabinowitsch infected fertile eggs with avian tubercle bacilli. The eggs were incubated. One hatched; the chick died of tuberculosis in 75 days. From this chain of evidence there would seem to be little

¹ *Rept. Vet. Director General of Canada*, 1912, p. 83.

doubt but that the eggs from diseased birds may serve to introduce the disease into healthy flocks. The purchaser of cattle is able to protect his herd from infection by the intelligent use of the tuberculin test. The uselessness of the subcutaneous test for fowls has been shown by Ward and by Edwards. It is also claimed¹ that neither tuberculin from avian or mammalian bacilli will produce a reaction when dropped into the conjunctival sac or applied to the scarified skin. Dr. Van Es² of the North Dakota

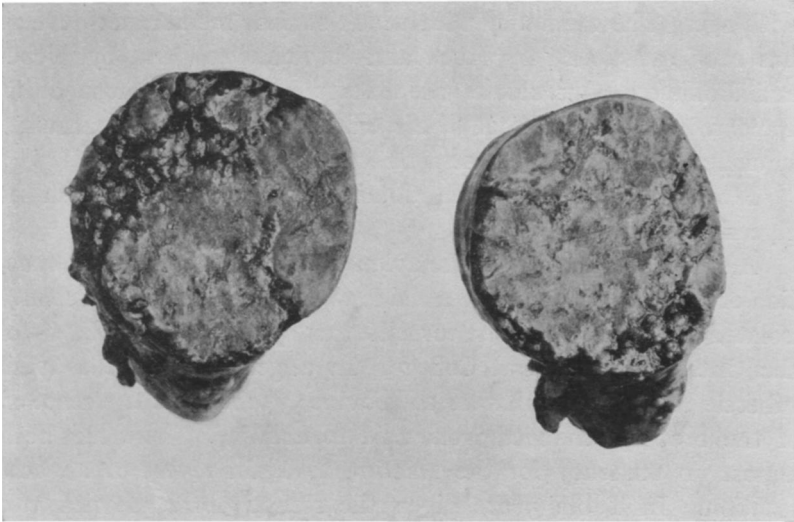


FIG. 3.—Tubercular spleen. The small amount of normal tissue is to be noted.

Experiment Station has made a number of tests of chickens by injecting avian tuberculin into the skin of the comb or wattles (intradermal test). Following his example two tests have been made by us on hens from a diseased flock. One reacted, the other did not. The results of the postmortem were in accord with the results of the test.

The avian tubercle bacillus.—Cultures directly from the tissues of hens have been isolated in nine of the 10 attempts made. Dorset's egg medium and glycerin agar have been used with success.

¹ Hutyra and Marek, *Pathology and Therapeutics of the Diseases of Domestic Animals*, translated by Mohler and Eichhorn, 1912, I, p. 614.

² Unpublished data.

The avian bacillus differentiates itself from the mammalian tubercle bacillus by the rapidity of growth on culture media, and the more pronounced color, yellow to pink, on solid media. In liquid media the growth is at first confined to the bottom, later a uniform turbidity is produced, and then the growth is again confined to the bottom, often forming a zoöglea-like mass, the broth remaining perfectly clear. If the depth of the fluid is not too great, the growth extends up the side of the flask until the surface is reached, when it spreads in a thin veil-like membrane which becomes thicker and wrinkled with age. The constancy with which surface membranes develop has been questioned by Koch and Rabinowitsch and by Weber and Bofinger. Our results are in agreement with those of O. Bang¹ who states that all of his cultures developed membranes, some appearing suddenly when the culture was old.

Meager growth has been obtained on media to which no glycerin was added.

A study of the effect of the avian tubercle bacillus on the reaction of glycerin broth has been made with a number of the cultures isolated as well as with one furnished us by Dr. S. F. Edwards of the Ontario Agricultural College. In a general way the avian tubercle bacillus is similar to the bovine type in that the reaction of the broth is changed from acid to alkaline. There has been almost no tendency for the reaction to again revert to an acid reaction. In Table 3 are given the results obtained with two

TABLE 3.
ALKALI PRODUCTION BY AVIAN TUBERCLE BACILLI.

Days Incubation	Culture 425.25	Check Flask	Days Incubation	Culture 425.15	Check Flask
	Per cent	Per cent		Per cent	Per cent
9.....	+1.55	+1.4	9.....	+1.5	+1.4
16.....	+1.0	+1.4	16.....	+1.0	+1.4
24.....	+1.2	+1.55	24.....	+0.95	+1.55
39.....	+1.0	+1.4	39.....	+0.55	+1.4
46.....	+1.0	+1.3	46.....	+0.55	+1.3
52.....	+0.75	+1.35	52.....	0.00	+1.35
59.....	+0.60	+1.35	59.....	0.00	+1.35
66.....	+0.80	+1.45	66.....	0.00	+1.45
73.....	+0.5	+1.4	173.....	0.00	+2.00
87.....	0.0	+1.8			
94.....	0.0	+1.8			

trials which are typical of all our results. Uninoculated flasks of the medium were incubated with the inoculated flasks. Dur-

¹ *Centralbl. f. Bakteriöl.*, I, Orig., 1906, 43, p. 34.

ing the long period of incubation some desiccation took place; this accounts for the increase in acidity of the uninoculated flasks.

The determinations were made by diluting 5 c.c. of the culture, after heating the same in the steamer, with 45 c.c. of distilled water that had just been boiled, and titrating with N/20 NaOH, using phenolphthalein as an indicator. In no case has any marked degree of alkalinity been produced, the solution showing but a faint pink on addition of the indicator.

The avian tubercle bacillus stains more easily than does the human or bovine organism. A cold aqueous solution of methylene blue applied for a few moments imparts to the organisms sufficient color to render them easily visible. Preparations treated with 5 per cent nitric acid in 80 per cent alcohol after staining with carbol-fuchsin, and counter stained with methylene blue, show many cells with a blue color. Often the appearance may lead one to believe the culture to be impure, but subcultures on various types of media do not reveal any impurities.

In many ways the relation between certain of the acidfast, non-pathogenic bacilli and the avian tubercle bacillus seems to be much closer than between the avian and mammalian tubercle bacilli. This point will be discussed more in detail later.

Infection of mammals with avian tubercle bacilli.—The results obtained by previous investigators with guinea-pigs have been contradictory. It seems probable that some of the observations have been incorrect since death may be produced with no macroscopic lesions, but cultures, and microscopic examination may reveal a large number of tubercle bacilli in the various organs.

Moore¹ reports that guinea-pigs inoculated with avian tissues died in a very emaciated condition but showed no tubercular lesions. Edwards was unable to infect guinea-pigs with avian tissues. A portion of the guinea-pigs inoculated with pure cultures were killed in 100 days, and lesions were found. The remaining pigs were apparently healthy at the end of 10 months thus showing the disease to be of the non-progressive type. Straus and Gamaleia² were unable to produce anything but slight lesions. The same was

¹ *Jour. Med. Research*, 1904, 9, p. 521.

² *Arch. de méd. expér. et d'anat. path.* 1891, 3, p. 850.

and Washburn¹ were able to infect hogs by feeding avian tissues. In our work four hogs have been fed on avian tubercular tissues. They were killed about six months after the first feeding. The lesions in all were confined to the cervical and mesenteric lymph glands. It is an open question whether the avian bacillus can produce such an extensive form of the disease in hogs as to cause condemnation of the carcass, but it is certain that it might cause such parts as the head to be rejected. Thus the disease becomes of economic importance other than in the poultry industry. Two hogs have been fed with pure cultures of the avian bacillus. That infection resulted from the inoculation has been shown by the tuberculin test with avian tuberculin. The temperature records of the tests are given in Table 4.

TABLE 4.
TUBERCULIN TESTS ON HOGS FED AVIAN TUBERCLE BACILLI. AVIAN TUBERCULIN.

Pre-Injection Temperatures	Hog 1	Hog 2	Hog 3
8 A.M.	102.2	102.4
10 A.M.	102.2	102.0	104.1
12 M.	102.3	102.4	103.0
2 P.M.	102.3	102.6	102.7
4 P.M.	102.6	102.6	103.0
6 P.M.	103.0	102.6	103.3
8 P.M.	102.6	102.0	102.0
Post-Injection Temperatures			
6 A.M.	103.0	106.8	106.4
8 A.M.	103.2	106.8	104.5
10 A.M.	104.4	107.2	106.1
12 M.	105.4	105.6	105.4
2 P.M.	105.4	105.8	105.0
4 P.M.	105.8	105.3	104.7
6 P.M.	105.8	105.2	104.0

Hogs 1 and 2 were fed four times with contaminated food, beginning August 15 and ending October 7, 1912, and the tests were made three months later. The high thermal reaction persisted for 36 hours and was accompanied by a severe constitutional reaction and swelling at the point of inoculation. Hog 3 was injected with cultures about three months before the test was made. Neither the thermal nor constitutional reaction was as marked in this case.

The two hogs fed have grown rapidly and show no evidence

¹ Circular 201, Bureau of Animal Industry, U.S. Dept. of Agriculture, 1912.

of a progressive type of the disease. We purpose to keep them for a longer period.

As a check on the test, two healthy hogs were injected with avian tuberculin. No thermal or constitutional reaction resulted.

Relationship of the avian tubercle bacillus to the mammalian types.—Most of the writers on avian tuberculosis have considered the question of the identity of the avian bacillus and the organisms from cattle and man. The usual conclusion has been that the avian organism represents a variety, caused by the adaptation of the organism to its environment, and that both the avian and mammalian are to be classed as one kind. It seems to the writers that there is very little ground for such an assertion. Culturally the avian organism is quite different from the mammalian types, differentiating itself by the rapidity and profuseness of growth and by the moist growth easily emulsified. The pigment development is marked and the growth at the bottom of the liquid media is certainly very different from the ordinary tubercle bacillus. It stains more easily and retains the stain less tenaciously than the mammalian types. Those who have held that all belong to one kind, based their conclusions largely on the similarity of the immunity reactions.

Recent work indicates that the acidfast bacilli represent a group with many properties in common and that there is more or less interchangeability in their immunity reactions. Wills¹ studied the tubercle bacillus, the leprosy, timothy grass, urine and blind worm organisms by means of the complement fixation test. He found that the serum of an animal infected with tubercle bacilli would give a reaction when any of the above-mentioned organisms were used as antigen in the complement fixation test. He believes that all the acidfast organisms have definite substances, common to all, and that these bodies are concerned in the production of antibodies. He found a quantitative not a qualitative difference in the organisms employed. Thwort² studied by means of the agglutination and complement fixation reaction, the relation of the organism causing Johne's disease or chronic dysentery in

¹ *Centralbl. f. Bakteriol.*, I, Orig., 1911, 61, p. 37.

² *Centralbl. f. Bakteriol.*, I, Orig., 1912, 66, p. 316.

cattle, to other acidfast organisms. Animals were inoculated with human, bovine, and avian tubercle bacilli, with Johne's bacillus and with *B. Phlei*, a non-pathogenic organism. The result of the work showed the specificity of the tests to be small. A serum that gave a positive result with its homologous organism was usually positive with the others.

Little has been done by us along similar lines but the results have been confirmatory of those of Wills and of Thwort. The hogs to which avian tissues or pure cultures were fed have been tested with tuberculin made with mammalian cultures. The first two tested gave no reaction whatever. Hogs 1 and 2 in Table 4 were retested 80 days later using ordinary tuberculin. The results of the test are given in Table 5.

TABLE 5.
TUBERCULIN TESTS ON HOGS FED AVIAN TUBERCLE BACILLI. ORDINARY
TUBERCULIN.

Pre-Injection Temperatures	Hog 1	Hog 2
10 A.M.	103.6	103.0
12 M.	102.5	102.2
2 P.M.	102.4	102.4
4 P.M.	102.6	102.3
6 P.M.	103.0	102.7
8 P.M.	102.6	101.8
Post-Injection Temperatures		
6 A.M.	102.4	105.8
8 A.M.	103.4	103.8
10 A.M.	104.5	104.3
12 M.	103.8	103.6
2 P.M.	103.1	102.6
4 P.M.	103.6	103.2
6 P.M.	103.5	102.3

It is to be noted that the thermal reaction was much less than in the previous test made with avian tuberculin and that the fever was of short duration. No swelling at the point of injection or constitutional reaction was noted. We have been unable to kill tubercular hens by the injection of ordinary tuberculin. As high as 6 c.c. of concentrated tuberculin injected into the peritoneal cavity of a hen, later shown to have generalized tuberculosis, produced but a slight effect. Guinea-pigs shown by postmortem examination to have extensive lesions produced by avian bacilli were not killed by 2 c.c. of a tuberculin, 0.1 c.c. of which was sufficient

to kill guinea-pigs infected with mammalian tubercle culture. Reeser¹ concluded from his work that tuberculin made with avian cultures was useless in testing cattle. It was suggested by O. Bang that avian tuberculin could be used as a diagnostic agent for Johne's disease. In some cases the results of the postmortem examination has agreed with the test, in others not.

Many trials have been made to infect hens by feeding sputum containing tubercle bacilli. A few have reported successful results, but the great majority of trials have given negative results. In our work a fowl was fed a mixture of meal and sputum 25 times between June 23 and October 7. The sputa used were from various sources. Two other fowls were fed like material 20 times during a period of three months. The results in all cases were negative. In a number of the outbreaks brought to our attention it had been thought by the veterinarian that the cause was sputum to which the birds may have had access, since there were cases of tuberculosis in the families.

It seems quite probable that the acidfast bacilli represent a great natural group, that the great majority are saprophytic forms, occurring in the animal body, while a few are pathogenic, the most prominent being the mammalian tubercle bacillus, the leprosy organism and Johne's bacillus and that the avian organism bears the same relation to the true tubercle bacillus as does the leprosy organism and Johne's bacillus. As is well known some of the saprophytic forms may produce lesions in guinea-pigs that can not be differentiated from the true tubercle by a macroscopic or microscopic examination; especially is this true when they are introduced with a considerable amount of foreign matter such as butter fat.

It is of course possible that the avian organism may be capable of producing lesions, if not a more serious form of the disease in man. There is some evidence to indicate this, but it remains certain that the great importance of the disease is as an economic factor in the poultry industry. This factor is destined to become of greater importance unless the poultrymen recognize more than at present the necessity of purchasing birds and eggs from known healthy flocks.

¹*Centralbl. f. Bakteriöl.*, I, Orig., 1908, 46, p. 159.

SUMMARY.

1. Avian tuberculosis is widespread in the United States and Canada. Undoubtedly it is increasing rapidly in extent.

2. Avian tuberculosis is primarily a disease of the abdominal cavity. The liver was tubercular in 97 per cent of the cases examined, the spleen in 93 and the intestines in 60 per cent.

3. It is probable that the diseased bird is the important factor in the transmission of the disease from flock to flock. Eggs may also be a factor of some importance.

4. It seems probable that the avian tubercle bacillus is not a true tubercle bacillus, but rather bears the same relation to the bovine and human tubercle bacilli as do the organisms of leprosy and of Johne's disease. The acidfast bacilli represent a great natural group.